Limits to Phenotypic Plasticity: Flow Effects on Barnacle Feeding Appendages

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Phenotypic plasticity, the capacity of a given genotype to produce differing morphologies in response to the environment, is widespread among marine organisms (1). For example, acorn barnacles feed by extending specialized appendages (the cirral legs) into flow, and the length of the cirri is plastic: the higher the velocity, the shorter the feeding legs (2, 3). However, this effect has been explored only for flows less than 4.6 m/s, slow compared to typical flows measured at sites on wave-exposed shores. What happens at faster speeds? Leg lengths of Balanus glandula *Darwin, 1854, an acorn barnacle, were measured at 15 sites in Monterey, California, across flows ranging from 0.5 to 14.0 m/s. Similar to previous findings, a plastic response in leg length was noted for the four sites with water velocities less than 3 m/s. However, no plastic response was present at the 11 sites exposed to faster velocities, despite a 4-fold variation in speed. We conclude that the velocity at which the plastic response occurs has an upper limit of 2–4 m/s, a velocity commonly exceeded within the typical habitat of this species.*

Acorn barnacles provide an excellent opportunity for examining plastic response because they are sessile (and therefore cannot move in response to the environment), molt their exoskeleton (providing periodic opportunity for morphological change), and occur across a wide range of flow conditions. Helmuth and Denny (4) measured maximal wave-induced water velocities at 222 sites along the rocky intertidal shore at Hopkins Marine Station (HMS) in Pacific Grove, California (36°36′N, 121°53′W), and the variation

in velocity at each site was expressed as a function of offshore significant wave height (the average height of the highest one-third of waves). These measurements allowed us to select sites exposed to a range of wave-induced water velocities. Eleven sites, each 1.5 m above the mean lower low water (MLLW), were selected for collection of *B. glandula*. Because of the exposure of this shore, all HMS sites except one are subjected to water velocities greater than those encountered in previous studies on *B. glandula* (2, 3). Therefore, four additional sites were selected at the Monterey Wharf in Monterey, California (~2 km from HMS), where barnacles are subjected to a range of slower flows. At each site, 10 barnacles were collected, and the length of each cirrus was measured.

Offshore significant wave height was measured four times per day for 30 days prior to the barnacle collections, and the largest significant wave height occurring when the tidal height was greater than 1.5 m above MLLW was noted for each day. These data, in conjunction with the relationships measured by Helmuth and Denny (4), allowed us to estimate the daily maximal water velocities imposed at each collection site. Marchinko (3) found that transplanted specimens of *B. glandula* begin modifying their cirral length in response to their new environment somewhere between 7 and 18 days after first exposure. There was no evidence of alteration at day 7 and significant alteration by day 18, continuing through day 30. To incorporate this lag in response time, we used the water velocities recorded 10–30 days prior to sampling as an index of the flows to which the barnacles could have responded. Previous studies have examined only the relationship between cirrus length and average daily maximum velocity, although the barnacles could be responding to maximum velocity, average velocity, or some other aspect of flow. We employ both the average daily maximum (ADM) velocity and overall maximum (OM) velocity.
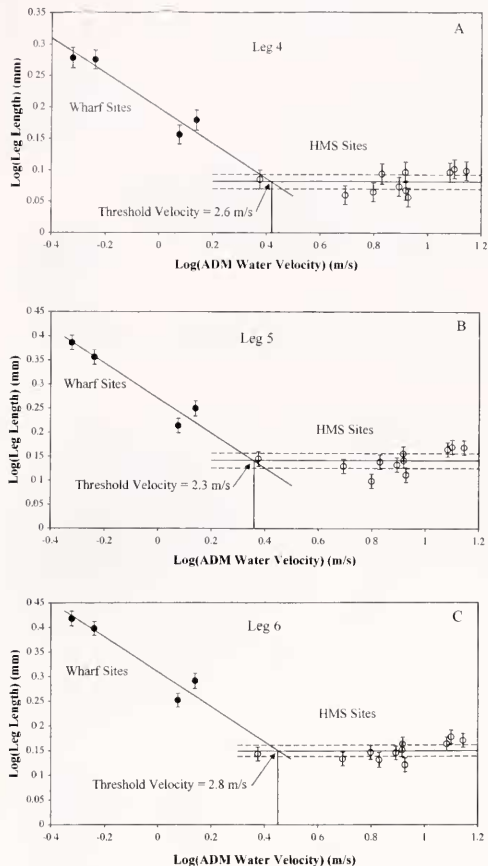
Figure 1. Average length of three feeding legs of *Balanus glandula* from 15 sites of differing wave exposure in Monterey Bay, California. Wave exposure was calculated as the average of daily maximum velocities encountered for 10–30 days prior to collection. Legs 4, 5, and 6 refer to the fourth, fifth, and posterior-most (sixth) pair of thoracic legs of these balanomorph barnacles. Leg lengths ($n = 10$ per site) were standardized to a common prosoma wet mass of 0.0079 g by ANCOVA. Reduced major axis regression lines are shown for the low-flow Wharf data. Lines representing the mean and 95% confidence intervals are shown for the HMS sites. (A) Average $\log_{10}$ length of leg 4. (B) Average $\log_{10}$ length of leg 5. (C) Average $\log_{10}$ length of leg 6. Reduced major axis regression statistics are given in Table 1. Error bars are standard errors of the log-transformed data.

Water velocities during high tide at the Monterey Wharf sites were measured using a Marsh-McBirney 511 electromagnetic flow meter. The probe was placed about 0.5 m seaward of the collection points, and the velocity was sampled every 30 s for 20 min at each site. The maximum velocity encountered was then used in conjunction with the co-occurring offshore wave height as a means of estimating maximal velocity. We assume that the velocity can be modeled using solitary wave theory (10), $U = k(gh)^{0.5}$ (where U is velocity, g is the acceleration of gravity, and h is offshore

All cirral legs (legs 4, 5, and 6) were significantly longer at the protected Monterey Wharf sites than at the exposed HMS sites. For example, the average length of leg 6 at the two wharf sites with ADM velocities less than 0.6 m/s was 2.56 mm, nearly 1.8 times that at HMS (avg. length = 1.42 mm), where ADM velocities exceeded 2.4 m/s. On the basis of evidence from previous studies (2, 3), we assume that this difference is due to a plastic response to flow. In contrast, the differences among the HMS sites were not significant, despite a range of velocities from 2.4 to 14.0 m/s. Furthermore, among the Monterey Wharf sites, there was a significant difference between the sites with lower velocities (0.48 and 0.58 m/s ADM) and higher velocities (1.19 and 1.38 m/s ADM).

Among the HMS sites, there was no significant correlation between leg length and either measure of water velocity ($P > 0.05$). In contrast, at the Monterey Wharf sites, leg length and water velocity were significantly correlated, and we explored this correlation using reduced major axis regressions for the logarithm of leg lengths *versus* the logarithm of either ADM or OM velocity (Fig. 1 and Table 1). We employed reduced major axis regression because the

significant wave height), and the constant of proportionality, k, was calculated for each site. Velocities could then be calculated for days 10–30 prior to barnacle collection from average daily maximum offshore wave significant height measurements. Note that in the summer of 2002 (when barnacles were collected), wave heights in Monterey Bay were consistently small, and variation among days was minimal.

We collected 10 solitary, uncrowded specimens of *Balanus glandula* from rock surfaces within 6 cm of each dynamometer for 11 sites along HMS on 5 and 6 May 2002. At the Monterey Wharf, barnacles were collected from rocks at four sites with differing wave exposures on 20 July 2002. The staggered collection dates may have allowed seasonal variation in barnacle morphology, but we assume that any such variation is unlikely to account for the extreme differences in cirral lengths found between the sites. At each site, barnacles of various sizes were collected. All barnacles were dissected on the day of collection. The prosoma (the fleshy part of the body without the shell) was extracted, blotted with a paper towel, and weighed to the nearest 0.1 mg as a measure of wet body mass. The posterior three cirri (legs 4, 5, and 6) were then dissected from the left side of the prosoma. Both the exopodite and the endopodite of each leg were placed together on a microscope slide in a drop of saltwater. The slides were viewed and legs traced onto paper with the use of a camera lucida attached to the microscope. A calibration length measurement was taken using a stage micrometer. The traced length of each leg was then measured to the nearest micrometer by using a piece of cotton string placed against the tracing. The legs were measured from the base of the ramus to each tip, excluding the propodite segments.

Analysis of covariance was conducted using SYSTAT (Systat Software Inc., ver. 6.0 for Macintosh). All sites exhibited a common slope of mass *versus* leg length. Separate ANCOVA tests were done on each leg (4, 5, and 6). Least-square means of barnacle leg length for a standard body mass were computed by ANCOVA. A Tukey HSD test was performed to detect differences between velocities and least-square mean leg lengths. Reduced major axis regressions were calculated using the least squares standardized means (5). All regression and ANCOVA analyses were performed on $\log_{10}$-$\log_{10}$ transformed data, as per Arsenault *et al.* (3).

variables involved have different scales and are subject to measurement errors that are not easily specified (5). (Note that leg measurements were standardized to a common prosomal wet weight; see Table 2.) Among the Monterey Wharf sites, leg length is significantly negatively correlated with flow velocity, with slopes varying from -0.28 to -0.36 depending on the cirrus. The relationship using ADM velocities is shown in Figure 1. The intersection of the Wharf regression and HMS mean (with 95% confidence interval) using ADM velocity occurred between 2.0 and 3.1 m/s (average of intersection with the mean: 2.6 m/s) depending on the cirrus (Fig. 1). The intersection using OM velocity (not shown) occurred between 3.0 and 4.6 m/s (average of intersection with mean: 3.6 m/s). Because of the small number of data points ($n = 4$), we could not calculate confidence bands for the reduced major axis regressions.

The morphology of the cirri of *B. glandula* is likely to affect their ability to act as effective filters. The longer the legs, the more area they can subtend and the farther they can extend into the water flow, and therefore the more water they can potentially filter. However, if the legs grow too long, hydrodynamic forces could cause them to buckle or bend, and thereby to lose their functionality. The ability to vary leg lengths appropriately in response to different water velocities would therefore appear to be advantageous. Indeed, barnacles seem capable of adjusting leg lengths within one or two molts of exposure to different wave velocities (3), and Arsenault *et al.* (2) suggest that this "tuning" results in a precise power relationship between leg length and water velocity. Our results suggest, however, that there is a threshold water velocity ($\sim$2.6 m/s using ADM velocity, $\sim$3.6 m/s using OM velocity) above which barnacles cease responding plastically to flow. Above this velocity, the large

Table 1

Reduced major axis linear regression equations for the average $\log_{10}$(leg length) (mm) of Balanus glandula as a function of the average $\log_{10}$ of two measures of water velocity (m/s) under breaking waves

Trait	Regression statistics			
	slope	intercept	r^2	P
A. Average daily maximum water velocity (ADM)				
leg 4	-0.279	0.198	0.9148	<0.001
leg 5	-0.362	0.271	0.9146	<0.001
leg 6	-0.354	0.310	0.901	<0.001
B. Overall maximum water velocity (OM)				
leg 4	-0.279	0.245	0.9148	<0.001
leg 5	-0.362	0.332	0.9146	<0.001
leg 6	-0.354	0.370	0.901	<0.001

Regressions for ADM and OM velocity have identical slopes because (for our method of estimating velocity at these sites) OM velocity is a constant multiple of ADM velocity. Data are shown only for the Monterey Wharf sites. No significant correlations were found among the sites at Hopkins Marine Station ($P > 0.05$). Sample size (n) for each leg is 4.

Table 2

Analysis of covariance results for measurements of Balanus glandula

Source of variation	df	Mean-square	F	P
Log(ramus length of fourth thoracic leg)				
Field site	14	0.052	22.876	<0.001
Covariate(log(prosoma wet mass))	1	0.392	172.237	<0.001
Residual	134	0.002		
Log(ramus length of fifth thoracic leg)				
Field site	14	0.069	31.817	<0.001
Covariate(log(prosoma wet mass))	1	0.359	166.552	<0.001
Residual	134	0.002		
Log(ramus length of sixth thoracic leg)				
Field site	14	0.082	40.281	<0.001
Covariate(log(prosoma wet mass))	1	0.369	180.598	<0.001
Residual	134	0.002		

drag forces experienced might not allow legs of any practical length to act as effective filters, and a plastic response would lose its advantage. Previous studies have reported that acorn barnacles adjust their feeding behavior across a range of low water velocities (0–0.15 m/s [6, 7]) and maintain their feeding activity at water velocities of at least 0.25 m/s (8, 9), but we know of no direct observations at greater velocities. Our results suggest that acorn barnacles on wave-exposed shores may be able to feed only during the relatively slow backwash as waves recede.

Arsenault *et al.* (2) propose that the tight relationship between leg length and water velocity might allow barnacles to be used to measure local wave exposure. Our results suggest that barnacle leg length can, indeed, be a reliable indicator of wave exposure, but only for sites at which the ADM water velocity is less than about 3 m/s. Note also that the exponents of the power relationship found by Arsenault *et al.* (2) (-0.32 to -0.43) are slightly different from those found here (-0.28 to -0.36). This disparity could be an artifact of the small number of data points ($n = 6$, Arsenault *et al.* [2]; $n = 4$, this study), but also could possibly be accounted for by differences in mean barnacle size (0.0219 g, Arsenault *et al.* [2]; 0.0079 g, this study), method used to estimate water velocity, or substantial latitudinal difference in collection site. Therefore, local calibration might be necessary if barnacles are to be used as "exposure meters."

Studies to date have examined only the ultimate relationship between maximum velocities and cirral length. As with any correlation, further research is needed to elucidate the mechanisms that account for the relationship.

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